Enzymes involve in Replication

① Initiator protein → Prokaryotic, Eukaryotic

② Helicase

③ Topoisomarase

④ Primase

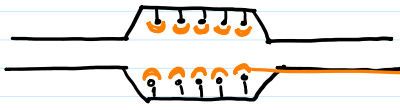
⑤ DNA Polymarase

⑥ SSB

* Primase

- Add short RNA primer (2-10 nt)
- DNA Dependent RNA Polymarase
- 3' → 5' Exonuclease activity or Proof reading activity - nt
- Prokaryotic Primase - DNA G
- Eukaryotic Primase - Primase Subunit is Associated with DNA Pol. → Pol. α

⑤ Single Stranded Binding Protein [SSB] ⇒



- Binds with Unwinded Single Stranded DNA via weak interaction

- SSB → +nt in Prokaryotes
- Replication protein A (RPA) → +nt in Eukaryote

⑥ DNA polymarase

- DNA dependent DNA polymarase

- Prokaryotic DNA Polymarase -

① – DNA Pol. I

② – DNA " II → Base Excision Repair

③ " " III

④ " " IV

⑤ " " V

→ Error prone polymarase

Pol - I, III → main DNA Pol.

DNA Pol. IV

↓

Gene: Din B

DNA Pol. V

↓

Gene – umu C (monomer)
umu D (Dimer)

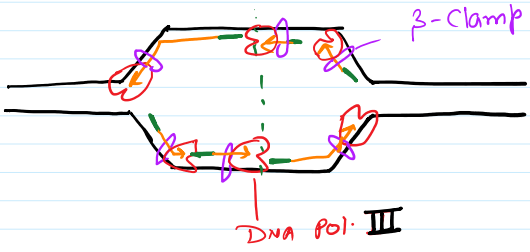
↓

- are inducible Polymarase
- Expresses during excessive DNA Damage
- do not Contain proof reading activity
- Belongs to Y family Polymarase

DNA Pol. I & III → most active DNA Pol. in Replication

RNA primer is added by Primase

Primase ← DNA Pol. III



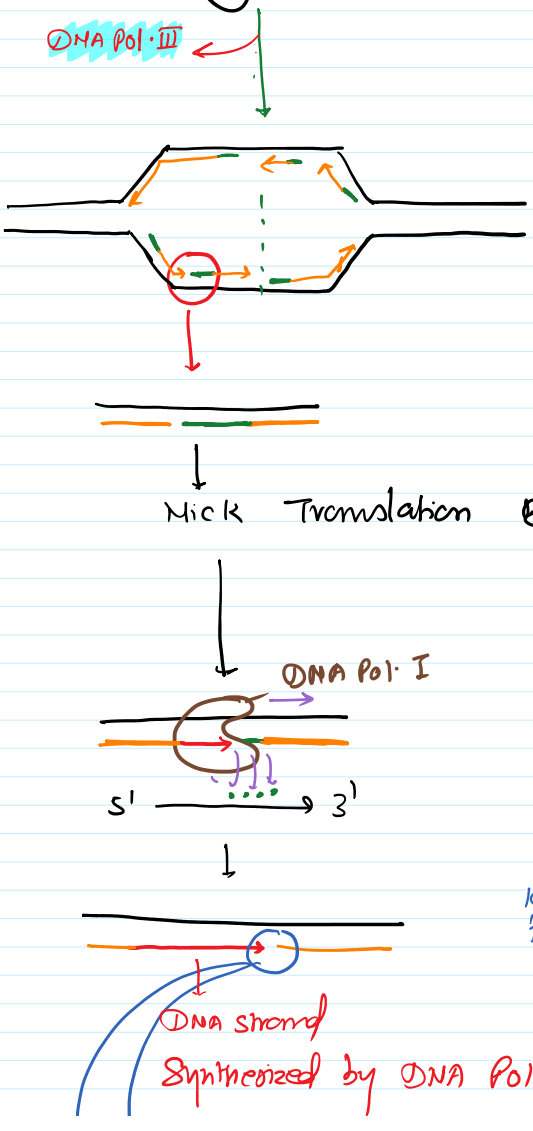
DNA Pol. III

- is a processive enzyme
  - Reason - β-Clamp is associated with Pol.
    - ↓
    - loded by Clamp loder (γ-subunit)
      - use ATP for loading

Replication Error Rate - = $1/10^5$ ✓

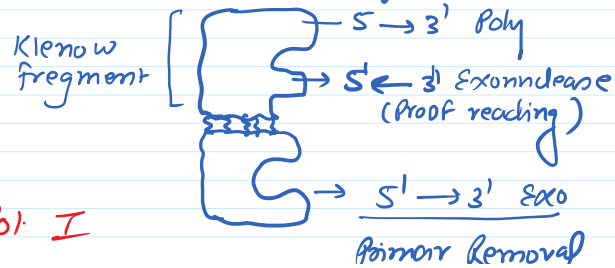
- 5' → 3' Polymarase activity
- if Exonuclease activity (3' → 5') is +nt → Error Rate = $1/10^7$
- if mismatch Repair System is working = Error Rate = $1/10^{10}$

Bacterial genome size = 4.6×10^6 bp



- Primer Remove
  - By 5' → 3' Exonuclease
- Add New nt.

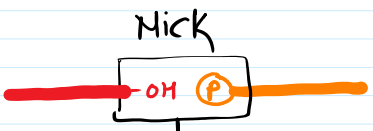
5' → 3' Polymarase activity



DNA Strand Synthesized by DNA Pol. I

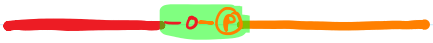
→ 5' → 3' Exo
Primer Removal activity

DNA Pol I Klq
Kornberg Enzyme



DNA ligase (For Nick Sealing)

Formation of Phosphodiester Bond
By using ATP / NAD
Eukaryote
Prokaryote



Phosphodiester Bond

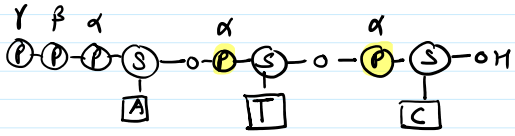
DNA Pol. I, also used in Probe Labelling

Probe Labelling

5' End Labeling

γ-P Radiolabelling

Compleat Labelling



α-P Should radiolabelled

Eukaryotic DNA Polymarase

DNA Pol.	Subunit	Function
Pol. α	4	Primer Synthesis during Replication
Pol. β	1	Base Excision Repair
η γ	3	mitochondrial DNA Replication
Pol. δ	2-3	Lagging Strand DNA Synthesis & Nt & Base Excision Repair
ε	4	Leading Strand Synthesis

		Repair
ε	4	leading Strand Synthesis Nt & Base Excision Repair
θ	1	Cross Strand Repair
✓ ζ	1	TLS [Trans lesion synthesis]
Pol. λ	1	meiosis Associated DNA Repair
Pol μ	1	Somatic Hyper mutation
κ	1	TLS
ν	1	TLS
Rev-1	1	TLS
ι	1	TLS

Nuclear Replicative DNA Pol.

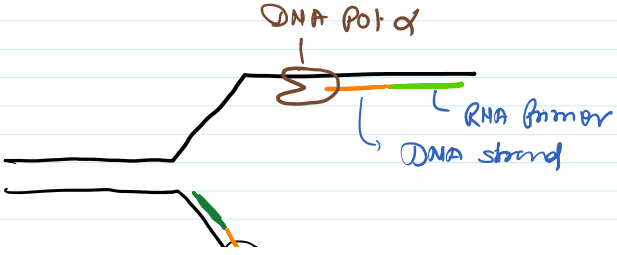
Pol. α, δ, and ε

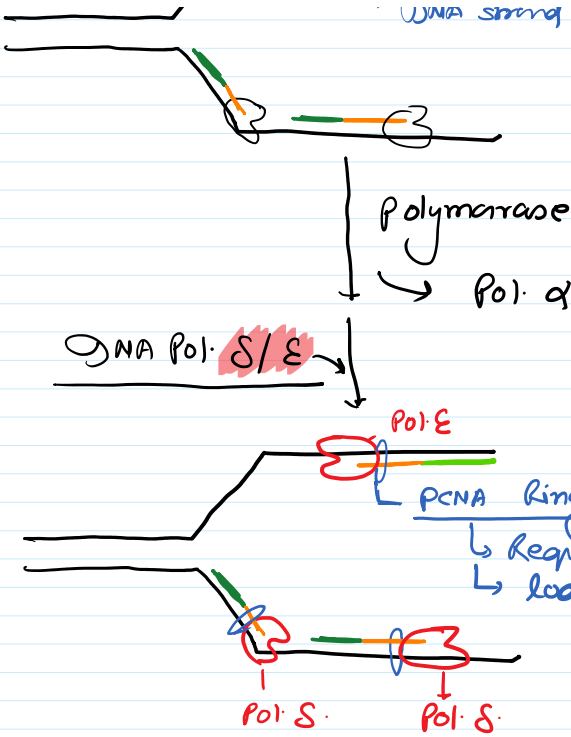
- 5' ⟶ 3' Polymarase activity
- 3' ⟶ 5' Exonuclease in [except α]

* In DNA Pol. α. → Synthesis of Short RNA Primer
- during its primase activity There is no proofreading activity
- after addition of short primer Pol. α Extend RNA primer with DNA nt.

↓

add ≈ 30 nt & then Dissociated.

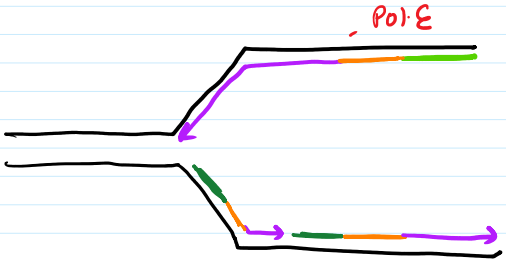


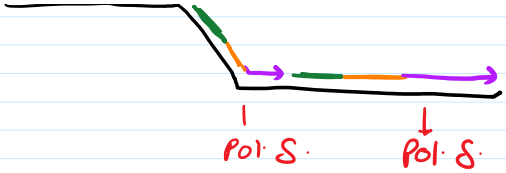


PCNA Ring [act as β- clamp]
↳ Required for Processivity
↳ loaded by RFC-40 Protein
↓
- Replication factor C-40 protein
↓
- act as clamp loader
- Required ATP

DNA Pol. δ/ε

- Extend 3' OH end on short segment that is added by DNA Pol. α
- Are Processive Enzyme
  - due to presence of PCNA Ring + RFC Protein
    ↓
    Also involve in
    - Repair mechanism
    - Chromosomal Remodelling
      Euchromatin ⇄ Heterochromatin
    - Also play role in Post Transcriptional modification





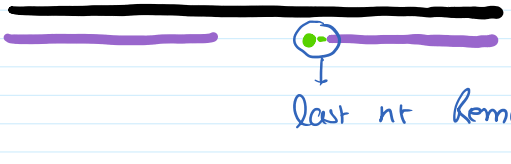
Primer Removal

(i) RNase H

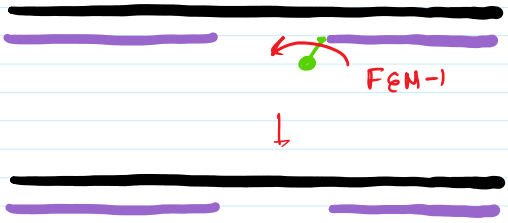
(ii) FEN-1 [Flap Endonuclease]

↓ ← RNase H [Recognize DNA-RNA hybrid]

↓ degrade RNA from Hybrid



Last nt Removed by FEN-1

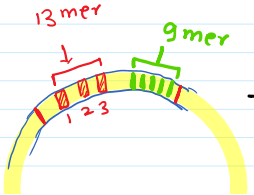


↓ Gap Filling done by Pol. δ/ε

↓ ← ligase (Nick Sealing)

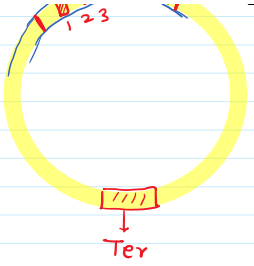
Prokaryotic Replication

Prokaryotic Genome – Circular

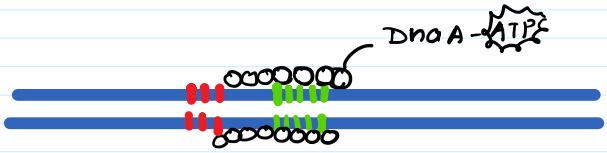


→ ori-c of E. coli

↓



AT Rich



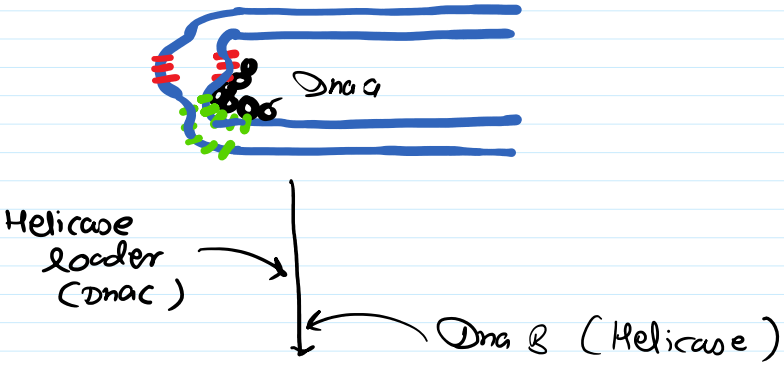
Binding of DnaA → Brings The Conformational change in DNA

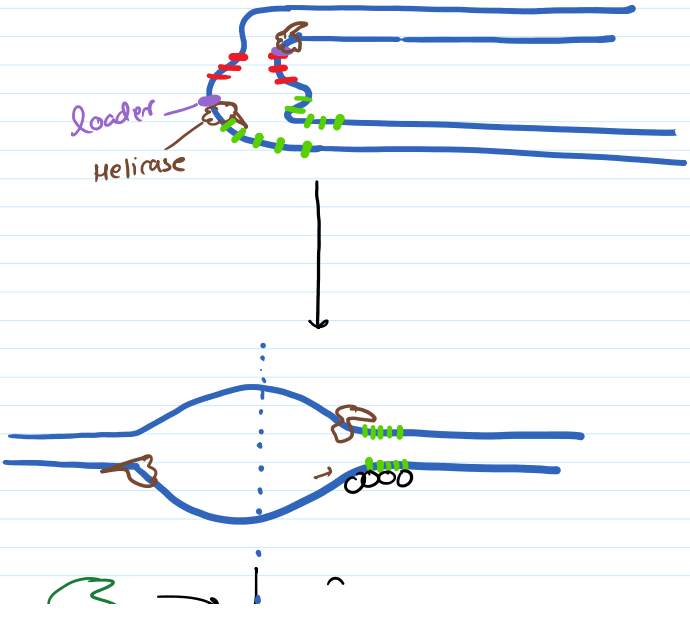
↓

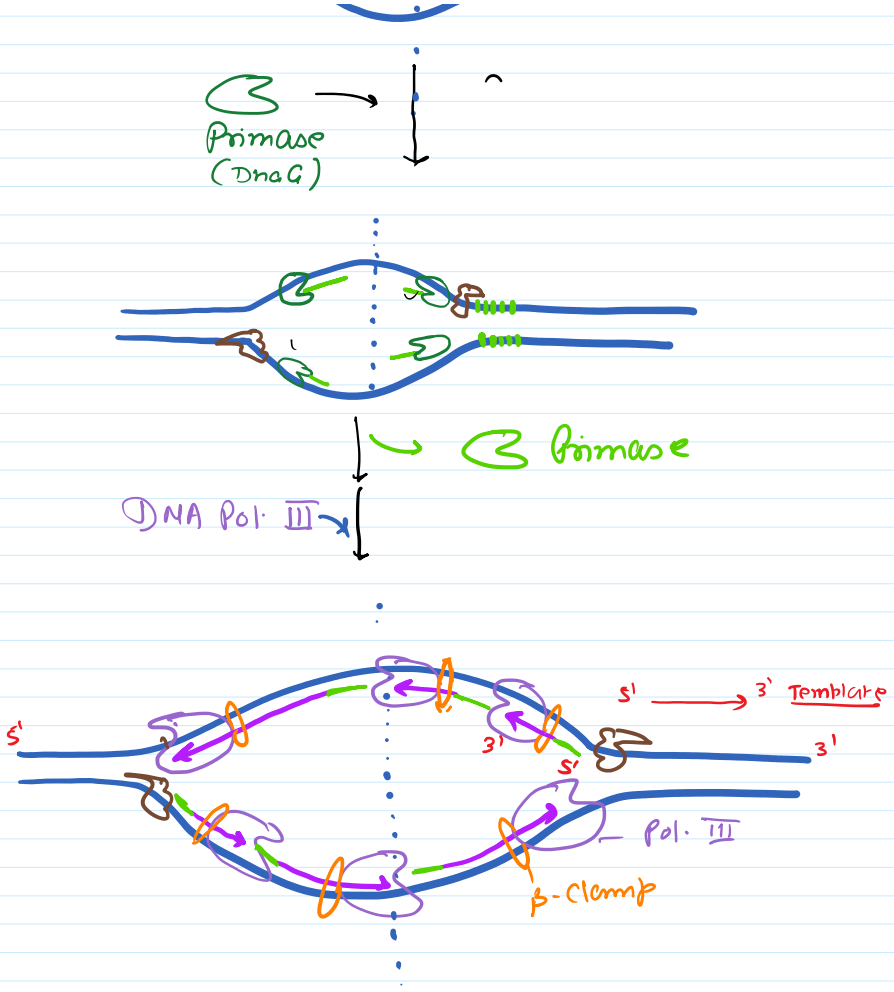
DNA Bending

↓

Melting of H-Bond at AT Rich Seq.







⇒ Prokaryotic DNA Replication speed = 1000 nt/sec

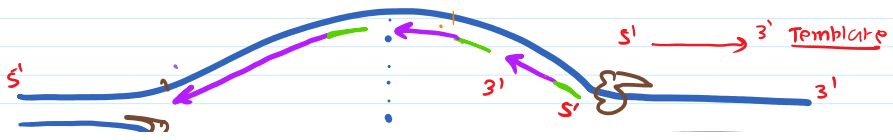
- Bidirectional Replication
- in Prokaryotes only 1 origin of Replication Seq. is present
- E. coli Genome size = 4×10^6 bp

Replication duration = $\frac{\text{Genome size}}{\text{Speed of Replication} \times (1) \times (2) \times 60}$

(1) → origin; (2) → Bidirectional Replication

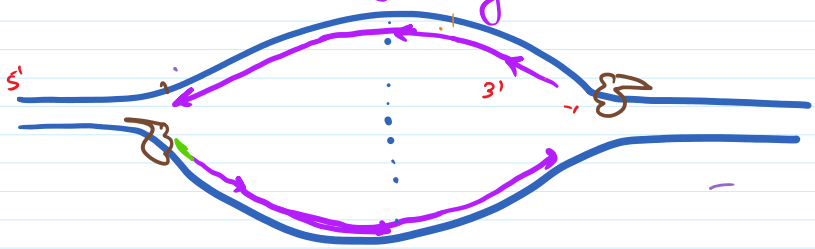
$$\frac{4 \times 10^6}{1000 \times 1 \times 2 \times 60}$$

$$= \frac{4 \times 10^2}{12} = \frac{400}{12} = 33.33 \text{ min}$$



Pol · I

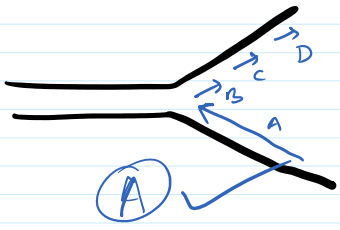
ligase

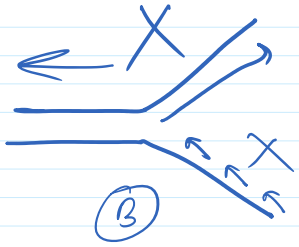


Replication mutation

Quick Stop gene
- Helicase
- Helicase loader
- DNA A
- DNA Pol·
- Primase

Slow Stop gene
- DNA Pol I
- ligase





⇒ in Prokaryotes DNA Replication → multiple Round.

↓

if there is mismatch in Newly synthesized DNA Strand

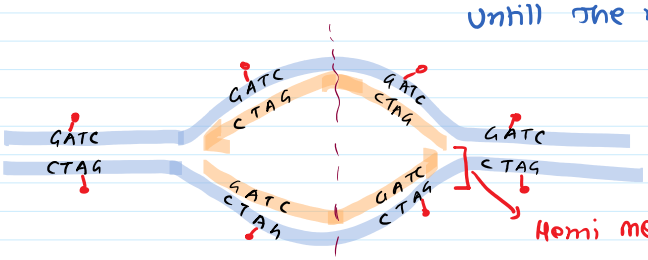
↓

Recognized by mismatch Repair System

↓ Repaired it

↓

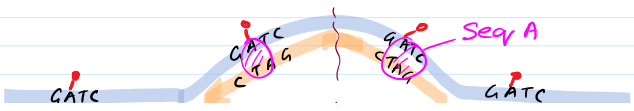
2nd Round Replication is not initiated Untill the mismatch is Repaired

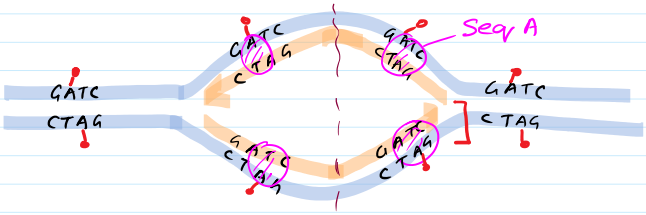


Hemi methylated Condition

Seq. A

Has ↑ affinity with Hemimethylated DNA





Dam methylase *

← Dna A [initiator]

* Seq A has ↑ affinity with Hemi methylated DNA

Seq·A Prevent Binding of Dam methylase and DnaA

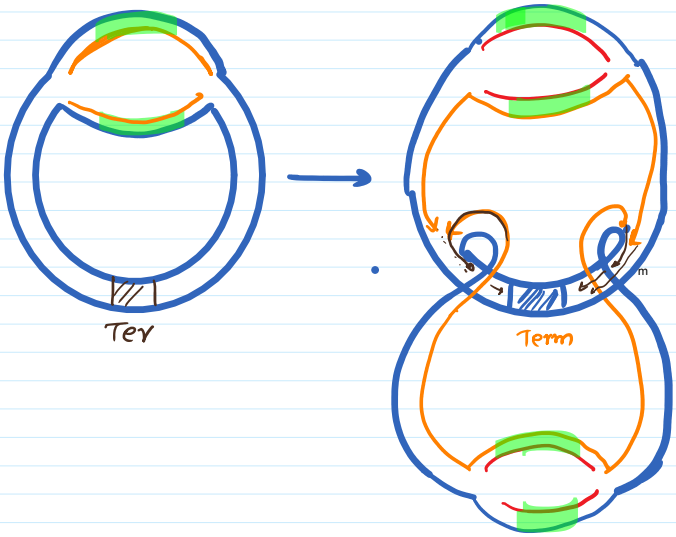
Additional time for mismatch Repair system

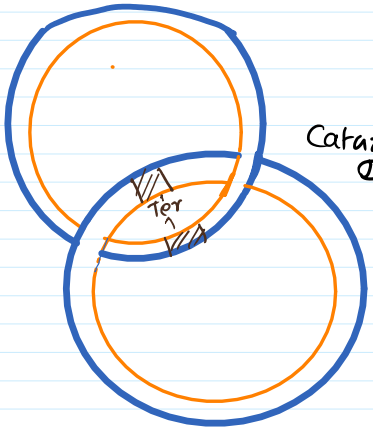
→ Seq·A

Dam methylase → methylation on New Strand (Both Strand is methylated)

DnaA → DnaA Recognize origin

2nd Round Replication start.

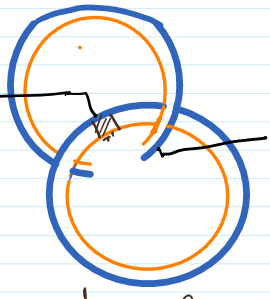




Catenated DNA

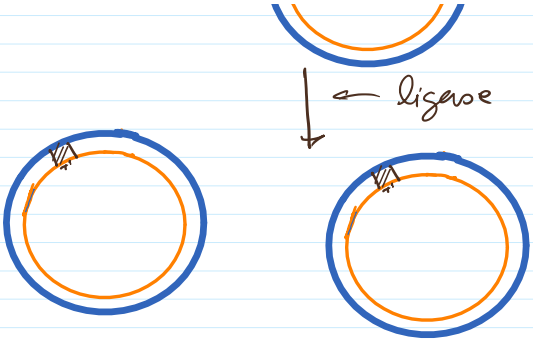
↓ decatenation by Topo II & IV

T-Strand transfer



G-Strand cut induced by Topo·II

↓ release


ligare